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In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

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Signed Andrew Gersey

Dated 3 March 2000

Patents Form 1/77

Patents Act 1977
(Ru[illegible]

The Patent Office

1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

10 JUL 1999

1. Your reference — PHM 99-095

2. Patent application number
(The Patent Office will fill in this part) — 9916099.6

3. Full name, address and postcode of the or of each applicant *(underline all surnames)* — Zeneca Limited
15 Stanhope Gate
LONDON
W1Y 6LN, GB

10 JUL 1999
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Patents ADP number *(if you know it)* — 6254007002

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention — CHEMICAL COMPOUNDS

5. Name of your agent *(if you have one)* — BILL, Kevin

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* — AstraZeneca PLC
Global Intellectual Property
Mereside, Alderley Park,
Macclesfield, Cheshire, SK10 4TG, GB

Patents ADP number *(if you know it)* — 4469847002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	30
Claim(s)	
Abstract	
Drawing(s)	

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Date
9 July 1999

12. Name and daytime telephone number of person to contact in the United Kingdom — Mrs Lynda May Slack 01625 516173

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

CHEMICAL COMPOUNDS

The present invention relates to compounds that are cysteine protease inhibitors and in particular compounds that are Cathepsin L inhibitors and or Cathepsin S inhibitors especially Cathepsin S inhibitors. The invention further relates to processes for their preparation, to intermediates useful in their preparation, to their use as therapeutic agents and to pharmaceutical compositions containing them.

Cysteine proteases are enzymes important in normal cell physiology, but they are also associated with several disease states including inflammation, metastasis, tissue damage following myocardial infarction, bone resorption and muscle wasting in dystrophic diseases.

Cathepsins B, H, K, L, N and S are cysteinyl proteases involved in normal protein degradation and are normally located in the lysosomes of cells. However, when these enzymes are found outside the lysosomes they have been implicated as playing a causative role in a number of disease states including bone resorption disease such as osteoporosis.

The number of people living to an old age has increased dramatically in recent years. This has been marked by an increase in the number of people having osteoporosis and other diseases associated with old age. Osteoporosis is accompanied by a high incidence of bone fracture resulting in many aged patients being confined to their beds. There is therefore a great need for a pharmaceutical composition to treat or prevent this disease.

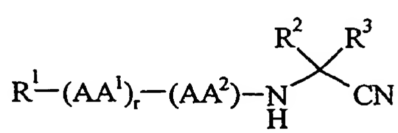
Living bone is continuously being remodelled and replenished by the process of resorption and deposition of the protein matrix and calcium minerals. These events are facilitated by the osteoclast, which has the ability to degrade and demineralise the bone, and the osteoblast which is responsible for new bone generation. In normal situations these processes are intimately linked resulting in little alteration of bone mass. However, pathological conditions exist in which there is an imbalance between their activity resulting in increased resorption of bone and the development of fragile and/or brittle bone structure, as seen during osteoporosis. While the exact mechanism for this resorption is not known, increased osteoclast activity, as realised by increased proteolytic activity, is a contributing factor, and selective inhibition of proteolytic action may result in the arrest or reversal of bone loss. The lysosomal cysteine proteinases, cathepsins B, H, K, L, N and S have been postulated as the proteinases that are responsible for osteoclast bone resorption, because of their ability to degrade insoluble type I collagens at low pH.

Cathepsins B, H, K, L, N and S have been further implicated as playing a causative role in other diseases such as rheumatoid arthritis, osteoarthritis, tumour metastasis, pneumocystitis, Crithidia fusiculata, malaria, trypanosoma brucei brucei, schistosomiasis, periodontal disease, metachromatic leukodystrophy and muscular dystrophy.

In recent years a number of synthetic inhibitors of cysteine proteases have been disclosed. US 5,055,451 discloses a series of peptidyl methyl ketones as thiol protease inhibitors; WO 95/15749 discloses peptidyl ketones with heterocyclic leaving groups as cysteine protease inhibitors; the *in vivo* inhibition of Cathepsin B by peptidyl (acyloxy) methyl ketones was discussed in *J. Med. Chem.* **1994**, *37*, 1833-40 and these types of compounds as inhibitors of cysteine protease inhibitors were also discussed in *J. Am. Chem. Soc.*, **1988**, *110*, 4429-4431; peptidyl diazomethyl ketones as specific inactivators of thiol proteinases was discussed in *J. Biol. Chem.*, **1981**, *256*, 4, 1923-8 and in *Methods in Enzymology*, **1981**, *80*, 820-5; the inhibiting activities of 1-peptidyl-2-haloacetyl hydrazines towards Cathepsin B and calpains was discussed in *Eur. J. Med. Chem.*, **1993**, 28 297-311 and peptidyl fluoromethyl ketones as inhibitors of Cathepsin B and the implication for treatment of Rheumatoid arthritis was discussed in *Biochemical Pharmacology*, **1992**, *44*, 6, 1201-7. A review of this prior art shows that there is a great need for a specific cysteine protease and especially a Cathepsin L inhibitor and or a Cathepsin S inhibitor.

The present invention discloses compounds with inhibitory activity of cysteine proteases and in particular of Cathepsin L and or Cathepsin S.

Accordingly the present invention provides a compound of formula **(I)**:



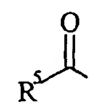
(I)

wherein:

r is 0 or 1;

$\mathbf{R^1}$ is optionally substituted benzyl where said optional substituents are chosen from one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, *N,N*-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, nitro, carboxy, carbamoyl, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl,

C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl, *N*-(C_{1-6}alkyl)sulphamoyl and *N,N*-(C_{1-6}alkyl)$_2$sulphamoyl or R^1 is a group of formula **(II)**:



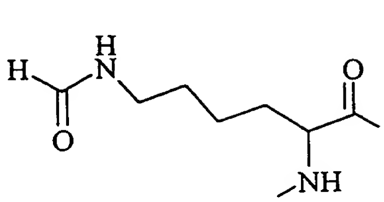
(II)

wherein $\mathbf{R^5}$ is C_{1-6}alkyl (optionally substituted with an optionally substituted phenyl, an optionally substituted 5 or 6 membered heteroaryl ring, optionally substituted phenoxy, optionally substituted phenylsulphonyl, optionally substituted C_{3-12}cycloalkyl or Het), C_{1-6}alkoxy, optionally substituted phenyl, optionally substituted naphthyl, optionally substituted 5 or 6 membered heteroaryl ring, optionally substituted C_{3-12}cycloalkyl, Het, optionally substituted phenylC_{1-6}alkoxy where said optional substituents are chosen from one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, *N,N*-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, nitro, carboxy, carbamoyl, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl, *N*-(C_{1-6}alkyl)sulphamoyl and *N,N*-(C_{1-6}alkyl)$_2$sulphamoyl;

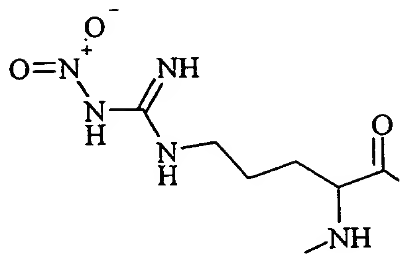
$\mathbf{R^2}$ is an optionally substituted 5 or 6 membered heteroaryl ring containing a maximum of four heteroatoms said optional substituents being chosen from one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, *N,N*-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, nitro, carboxy, carbamoyl, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl, *N*-(C_{1-6}alkyl)sulphamoyl and *N,N*-(C_{1-6}alkyl)$_2$sulphamoyl;

$\mathbf{R^3}$ is H; and

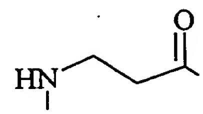
(AA^1) and **(AA^2)** are independently chosen from Ala, Arg, Cys, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, Val,



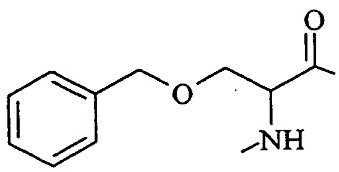
Lys(CHO),



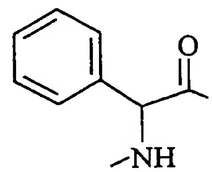
Arg(NO_2),



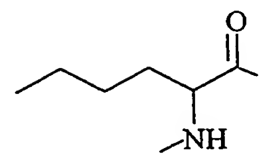
β-Ala,



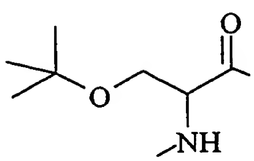
Ser(Bzl),



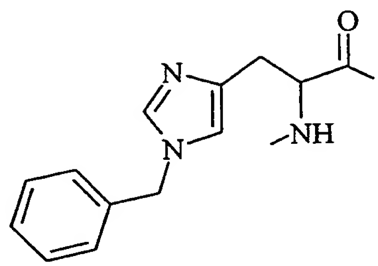
Ph-Gly,



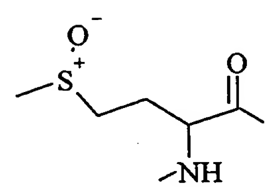
Nle,



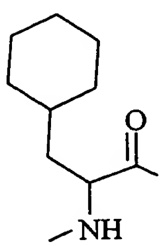
Ser(O^{t}Bu),



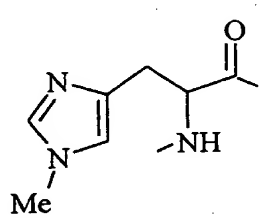
His(Bzl),



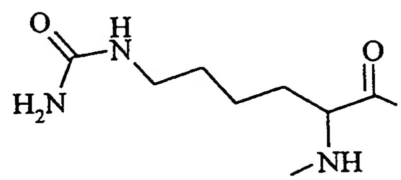
Met(O),



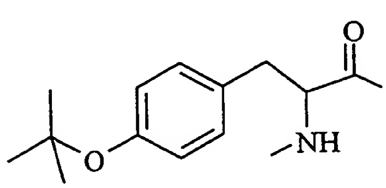
Cha,



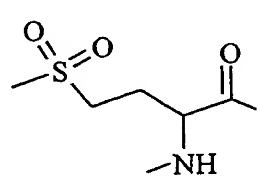
His(Me),



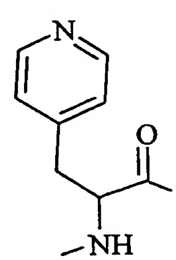
Cit,



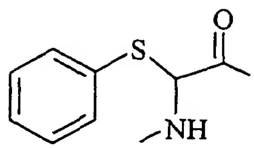
Tyr(^{t}Bu),



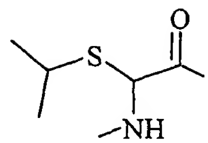
Met(O_2),



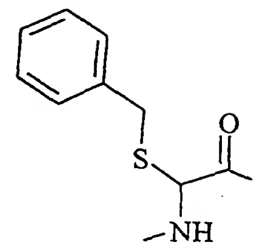
Pyr-Ala



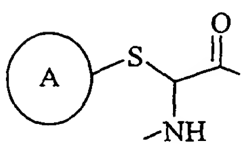
Phe(S),



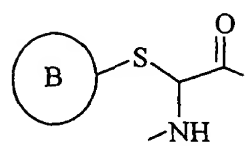
Leu(S),



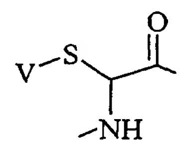
Phe(CH_2S),



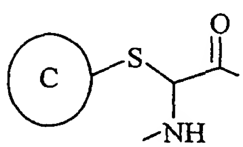
Cy(S)-Gly,



Hetar(S)-Gly,



alk(S)-Gly or



Het(S)-Gly;

wherein Ring A is C_{3-12}cycloalkyl, Ring B is a 5 or 6 membered heteroaryl ring, Ring C is Het, V is C_{1-6}alkyl excluding isopropyl, the nitrogen of the amino acid may optionally be alkylated with C_{1-6}alkyl and the phenyl group of Phe(S) and Rings A and B may be optionally substituted with one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, *N,N*-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, nitro, carboxy, carbamoyl, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl, *N*-(C_{1-6}alkyl)sulphamoyl and *N,N*-(C_{1-6}alkyl)$_2$sulphamoyl or the phenyl group of Phe(S) may be fused to another phenyl group to form a naphthyl group;

or a pharmaceutically acceptable salt thereof.

In this specification the term 'alkyl' includes straight chained and branched structures and ring systems. For example, C_{1-6}alkyl includes propyl, isopropyl, *t*-butyl, cyclopropyl and cyclohexyl. However, references to individual alkyl groups such as 'propyl' are specific for the straight chained version only, references to individual branched chain alkyl groups such as 'isopropyl' are specific for the branched chain version only and references to individual cycloalkyl groups such as cyclohexyl are specific to the cyclic groups only.

A similar convention applies to other radicals, for example "hydroxyC_{1-6}alkyl" includes 1-hydroxyethyl and 2-hydroxyethyl.

The term "halo" refers to fluoro, chloro, bromo and iodo.

"Het" means, unless otherwise further specified, a fully saturated monocyclic 5 - 8 membered heterocyclic ring, with up to 4 ring heteroatoms. Preferably these ring heteroatoms are selected from nitrogen, oxygen and sulphur. Examples of "Het" include pyrrolidinyl, imidazolidinyl, pyrazolidinyl, piperidyl, piperazinyl and morpholinyl.

Unless otherwise defined standard amino acid abbreviations are used. For example "Ala" refers to alanine and "Gly" refers to glycine.

"5- or 6- membered heteroaryl ring" means, unless otherwise further specified, a 5- or 6- membered ring that contains some degree of unsaturation, with up to four ring heteroatoms selected from nitrogen, oxygen and sulphur. Examples of "5- or 6- membered heteroaryl ring" include thienyl, furyl, imidazolyl, thiazolyl, pyrimidinyl, pyridinyl, pyrrolyl and pyrazolyl. Examples of "6 membered heteroaryl ring" include pyrimidinyl, pyridinyl, pyrazinyl and pyridazinyl. Examples of "5 membered heteroaryl ring" include thienyl, furyl, imidazolyl, thiazolyl, pyrrolyl and oxadiazolyl.

Examples of "C_{1-6}alkanoyloxy" are acetoxy and propionyloxy. Examples of "C_{1-6}alkoxycarbonyl" include methoxycarbonyl, ethoxycarbonyl, *n*- and *t*-butoxycarbonyl. Examples of "C_{1-6}alkoxy" include methoxy, ethoxy and propoxy. Examples of "C_{1-6}alkanoylamino" include formamido, acetamido and propionylamino. Examples of "C_{1-6}alkylsulphanyl" include methylthio and ethylthio. Examples of "C_{1-6}alkylsulphinyl" include methylsulphinyl and ethylsulphinyl. Examples of "C_{1-6}alkylsulphonyl" include mesyl and ethylsulphonyl. Examples of "C_{1-6}alkanoyl" include acetyl and propionyl. Examples of "C_{1-6}alkylamino" include methylamino and ethylamino. Examples of "*N,N*-(C_{1-6}alkyl)$_2$amino" include *N,N*-dimethylamino, *N,N*-diethylamino and *N*-ethyl-*N*-methylamino. Examples of "*N*-(C_{1-6}alkyl)carbamoylC_{1-6}alkyl" are 2-(methylamino)carbonylethyl and 3-(ethylamino)carbonylpropyl. Examples of "*N,N*-(C_{1-6}alkyl)$_2$carbamoylC_{1-6}alkyl" are 2-(dimethylamino)carbonylethyl and 3-(*N*-methyl-*N*-ethylamino)carbonylpropyl. Examples of "C_{2-6}alkenyl" are vinyl, allyl and 1-propenyl. Examples of "C_{2-6}alkynyl" are ethynyl, 1-propynyl and 2-propynyl. Examples of "*N*-(C_{1-6}alkyl)carbamoyl" are *N*-methylaminocarbonyl and *N*-ethylaminocarbonyl. Examples of "*N,N*-(C_{1-6}alkyl)$_2$carbamoyl" are *N,N*-dimethylaminocarbonyl and *N*-methyl-*N*-

ethylaminocarbonyl. Examples of "N-(C_{1-6}alkyl)sulphamoyl" are N-methylsulphamoyl and N-ethylsulphamoyl. Examples of "N,N-(C_{1-6}alkyl)$_2$sulphamoyl" are N,N-dimethylsulphamoyl and N,N-diethylsulphamoyl. Examples of "R^4C_{1-6}alkylsulphanyl" include R^4methylthio and 2-R^4ethylthio. Examples of "R^4C_{1-6}alkylsulphinyl" include R^4methylsulphinyl and 2-R^4ethylsulphinyl. Examples of "R^4C_{1-6}alkylsulphonyl" include R^4mesyl and 2-R^4ethylsulphonyl. Examples of R^4C_{2-6}alkenyl are 2-R^4vinyl and 3-R^4allyl. Examples of "C_{2-6}alkynyl" are 2-R^4ethynyl and 3-R^4propyn-1-yl. Examples of "N-(R^4C_{1-6}alkyl)carbamoyl" are R^4methylaminocarbonyl and 2-R^4ethylaminocarbonyl. Examples of "N-(HetC_{1-6}alkyl)carbamoyl" are morpholinomethylaminocarbonyl and 2-(piperidinoethyl)aminocarbonyl. Examples of "C_{3-12}cycloalkyl" are cyclopropyl, cyclopentyl and cyclohexyl.

Where optional substituents are chosen from "one or more" groups it is to be understood that this definition includes all substituents being chosen from one of the specified groups or the substituents being chosen from two or more of the specified groups. For example where optional substituents are chosen from one or more halo, C_{1-6}alkoxy and C_{1-6}alkyl, examples of possible combinations of substituents include 1) a bromo group, 2) two chloro groups, 3) a methoxy, ethoxy and propoxy substitutent, 4) a fluoro and a methoxy group, 5) a methoxy, a methyl and an ethyl group, and 6) a chloro, a methoxy and an ethyl group.

According to a further feature of the invention there is a compound of formula **(I)** wherein:

r is 0 or 1;

$\mathbf{R^1}$ is optionally substituted benzyl where said optional substituents are chosen from one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, N,N-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, nitro, carboxy, carbamoyl, N-(C_{1-6}alkyl)carbamoyl, N,N-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl, N-(C_{1-6}alkyl)sulphamoyl and N,N-(C_{1-6}alkyl)$_2$sulphamoyl or R^1 is a group of formula **(II)** wherein $\mathbf{R^5}$ is C_{1-6}alkyl (optionally substituted with an optionally substituted phenyl, an optionally substituted 5 or 6 membered heteroaryl ring, optionally substituted phenoxy or optionally substituted phenylsulphonyl), C_{1-6}alkoxy, optionally substituted phenyl, optionally substituted naphthyl, optionally

substituted phenylC_{1-6}alkoxy where said optional substituents are chosen from one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, *N,N*-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, nitro, carboxy, carbamoyl, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl, *N*-(C_{1-6}alkyl)sulphamoyl and *N,N*-(C_{1-6}alkyl)$_2$sulphamoyl;

R^2 is H, C_{1-6}alkyl [optionally substituted with one or more of hydroxy, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, R^4, R^4C_{1-6}alkylsulphanyl, R^4C_{1-6}alkylsulphinyl, R^4C_{1-6}alkylsulphonyl], or R^2 is C_{1-6}alkoxy [optionally substituted with one or more of C_{2-6}alkenyl, C_{2-6}alkynyl, R^4, R^4C_{2-6}alkenyl, R^4C_{2-6}alkynyl, Het and trifluoromethyl], or R^2 is C_{2-6}alkenyl, C_{2-6}alkynyl, C_{1-6}alkoxycarbonyl, carbamoyl, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, R^4, R^4S, R^4C_{1-6}alkylsulphanyl, *N*-(R^4C_{1-6}alkyl)carbamoyl, *N*-(HetC_{1-6}alkyl)carbamoyl, C_{1-6}alkanoylamino, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl wherein **R^4** is an optionally substituted phenyl, or an optionally substituted 5 or 6 membered heteroaryl ring containing a maximum of four heteroatoms said optional substituents being chosen from one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, *N,N*-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, nitro, carboxy, carbamoyl, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl, *N*-(C_{1-6}alkyl)sulphamoyl and *N,N*-(C_{1-6}alkyl)$_2$sulphamoyl;

R^3 is H or C_{1-6}alkyl; and

(AA^1) and **(AA^2)** are independently chosen from Ala, Arg, Cys, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, Val, Lys(CHO), Arg(NO_2), β-Ala, Ser(Bzl), Ph-Gly, Nle, Ser(O^{t}Bu), His(Bzl), Met(O), Cha, His(Me), Cit, Tyr(^{t}Bu), Met(O_2), Pyr-Ala, Phe(S), Leu(S) or Phe(CH_2S);

wherein the nitrogen of the amino acid may optionally be alkylated with C_{1-6}alkyl and the phenyl group of Phe(S) may be optionally substituted with one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, *N,N*-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, nitro, carboxy, carbamoyl, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl,

N-(C_{1-6}alkyl)sulphamoyl and N,N-(C_{1-6}alkyl)$_2$sulphamoyl or the phenyl group may be fused to another phenyl group to form a naphthyl group;
or a pharmaceutically acceptable salt thereof.

Preferred values for R^1, r, AA^1, AA^2, R^2 and R^3 are as follows.

In one aspect of the invention preferably $\mathbf{R^1}$ is optionally substituted benzyl where said optional substituents are chosen from one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, N,N-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, nitro, carboxy, carbamoyl, N-(C_{1-6}alkyl)carbamoyl, N,N-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl, N-(C_{1-6}alkyl)sulphamoyl and N,N-(C_{1-6}alkyl)$_2$sulphamoyl or R^1 is a group of formula **(II)** wherein $\mathbf{R^5}$ is C_{1-6}alkoxy, optionally substituted C_{3-12}cycloalkyl or optionally substituted phenylC_{1-6}alkoxy where said optional substituents are chosen from one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, N,N-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, nitro, carboxy, carbamoyl, N-(C_{1-6}alkyl)carbamoyl, N,N-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl, N-(C_{1-6}alkyl)sulphamoyl and N,N-(C_{1-6}alkyl)$_2$sulphamoyl.

Preferably R^1 is benzyl or a group of formula **(II)** wherein R^5 is C_{1-6}alkyl (optionally substituted with a 6 membered heteroaryl ring, phenyl, phenylsulphonyl or phenoxy optionally substituted with one or more halo), C_{1-6}alkoxy, phenyl (optionally substituted with one or more halo), naphthyl and phenylC_{1-6}alkoxy.

In another aspect of the invention preferably R^1 is benzyl or a group of formula **(II)** wherein R^5 is C_{1-6}alkyl (optionally substituted with a 6 membered heteroaryl ring, phenyl, phenylsulphonyl, C_{3-12}cycloalkyl or phenoxy optionally substituted with one or more halo), C_{1-6}alkoxy, phenyl (optionally substituted with one or more halo), naphthyl, C_{3-12}cycloalkyl, Het, and phenylC_{1-6}alkoxy.

More preferably R^1 is benzyl or a group of formula **(II)** wherein R^5 is methyl, methoxy, ethoxy, propoxy, ^{t}butoxy, phenyl, 2,4-dichlorophenyl, naphthyl, benzyloxy, pyridylmethyl, benzyl, 2,4,6-trichlorophenoxymethyl and phenylsulphonylmethyl.

In another aspect of the invention more preferably R^1 is benzyl or a group of formula **(II)** wherein R^5 is methyl, methoxy, ethoxy, propoxy, ^{t}butoxy, phenyl, 2,4-dichlorophenyl,

naphthyl, benzyloxy, pyridylmethyl, benzyl, 2,4,6-trichlorophenoxymethyl, phenylsulphonylmethyl, morpholino, cyclohexyl, cyclopentyl, cyclohexylmethyl and piperidino.

Particularly R^1 is a group of formula **(II)** wherein R^5 is methyl, ^{t}butoxy, benzyloxy and pyridylmethyl.

In another aspect of the invention particularly R^1 is a group of formula **(II)** wherein R^5 is methyl, ^{t}butoxy, benzyloxy, pyridylmethyl, morpholino, cyclohexyl, cyclopentyl, cyclohexylmethyl and piperidino.

More particularly R^1 is a group of formula **(II)** wherein R^5 is methyl, ^{t}butoxy, benzyloxy and 4-pyridylmethyl.

In another aspect of the invention particularly R^1 is a group of formula **(II)** wherein R^5 is morpholino, cyclohexyl, cyclopentyl, cyclohexylmethyl and piperidino.

In one aspect of the invention preferably r is 0.

In another aspect of the invention preferably r is 1.

Preferably AA^1 is Leu, Pyr-Ala and Phe wherein the nitrogen of the amino acid is optionally substituted with C_{1-6}alkyl.

More preferably AA^1 is Leu and the nitrogen of the amino acid is unsubstituted.

Preferably AA^2 is Phe, Leu, Ile, Tyr, Tyr(^{t}Bu), Val, Cha, Leu(S), Phe(S) and Phe(CH_2S) and the nitrogen of the amino acid is unsubstituted and the phenyl group of Phe(S) is optionally substituted with halo, C_{1-6}alkyl or is fused to another phenyl group to form a naphthyl group.

More preferably AA^2 is Tyr, Leu and Phe and the nitrogen of the amino acid is unsubstituted.

Preferred combinations of r, AA^1 and AA^2 are as follows.

When r = 0 preferably AA^2 is Phe, Leu, Ile, Val, Tyr, Tyr(^{t}Bu), Leu(S), Phe(S) and Phe(CH_2S) and the nitrogen of the amino acid is unsubstituted and the phenyl group of Phe(S) is optionally substituted with halo, C_{1-6}alkyl or is fused to another phenyl group to form a naphthyl group.

When r = 0 more preferably AA^2 is Tyr.

When r = 1 preferably AA^1-AA^2 is Leu-Leu, Pyr-Ala-Leu, Phe-Leu, Leu-Phe, Leu-Ile, Leu-Val, Leu-Cha and (N-Me)Leu-Leu.

When r = 1 more preferably AA^1-AA^2 is Leu-Leu and Leu-Phe.

In another aspect of the invention preferably **(AA^1)** and **(AA^2)** are both independently selected from Phe(S), Leu(S), Phe(CH_2S), Cy(S)-Gly, Hetar(S)-Gly, alk(S)-Gly and Het(S)-Gly wherein Phe(S) and Rings A and B may be optionally substituted as hereinbefore defined and wherein the phenyl group of Phe(S) may be fused to another phenyl group to form a naphthyl group.

In a further aspect of the invention preferably r is 0 and **(AA^2)** is selected from Phe(S), Leu(S), Phe(CH_2S), Cy(S)-Gly, Hetar(S)-Gly, alk(S)-Gly and Het(S)-Gly wherein Phe(S) and Rings A and B may be optionally substituted as hereinbefore defined and wherein the phenyl group of Phe(S) may be fused to another phenyl group to form a naphthyl group.

In a further aspect of the invention preferably r is 0 and **(AA^2)** is selected from Phe(S), Leu(S) and Phe(CH_2S) wherein Phe(S) may be optionally substituted as hereinbefore defined and wherein the phenyl group of Phe(S) may be fused to another phenyl group to form a naphthyl group.

In one aspect of the invention preferably **R^2** is C_{1-6}alkoxy [optionally substituted with one or more of C_{2-6}alkenyl, C_{2-6}alkynyl, R^4, R^4C_{2-6}alkenyl, R^4C_{2-6}alkynyl, Het and trifluoromethyl], or R^2 is C_{2-6}alkenyl, C_{2-6}alkynyl, carbamoyl, R^4, R^4S, R^4C_{1-6}alkylsulphanyl, C_{1-6}alkanoylamino, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl wherein **R^4** is an optionally substituted phenyl, or an optionally substituted 5 or 6 membered heteroaryl ring containing a maximum of four heteroatoms said optional substituents being chosen from one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, *N,N*-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, nitro, carboxy, carbamoyl, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl, *N*-(C_{1-6}alkyl)sulphamoyl and *N,N*-(C_{1-6}alkyl)$_2$sulphamoyl. Preferably R^2 is hydrogen, C_{1-6}alkyl [optionally substituted with C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphonyl or R^4], C_{1-6}alkoxy [optionally substituted with C_{2-6}alkynyl] and R^4- wherein R^4 is an optionally substituted phenyl or an optionally substituted 5 membered heteroaryl ring containing a maximum of four heteroatoms said optional substituents being chosen from one or more of C_{1-6}alkyl and halo.

More preferably R^2 is hydrogen, methyl, ethyl, propyl, isobutyl, furyl, thienyl, pyrazolyl (optionally substituted with one or more of methyl and bromo), imidazolyl, 1,2,4-

triazolyl, phenyl, benzyl, 2-methylthioethyl, methylthio, ethylthio, isopropylthio, mesylethy methoxy, ethoxy, isopropoxy and 2-propynyloxy.

Particularly R^2 is furyl, pyrazolyl (optionally substituted with one or more of methyl and bromo), imidazolyl, 1,2,4-triazolyl, benzyl, 2-methylthioethyl, isopropylthio, methoxy, isopropoxy and 2-propynyloxy.

More particularly R^2 is fur-2-yl, pyrazol-1-yl, 3,5-dimethylpyrazol-1-yl, 4-bromo-3,5-dimethylpyrazol-1-yl, imidazol-1-yl, 1,2,4-triazol-1-yl, benzyl, methylthioethyl, isopropylthio, methoxy, isopropoxy and 2-propynyloxy.

In another aspect of the invention preferably $\mathbf{R^2}$ is an optionally substituted 5 membered heteroaryl ring containing a maximum of four heteroatoms said optional substituents being chosen from one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, *N,N*-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, nitro, carboxy, carbamoyl, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl, *N*-(C_{1-6}alkyl)sulphamoyl and *N,N*-(C_{1-6}alkyl)$_2$sulphamoyl.

More preferably R^2 is thienyl, furyl and pyrazolyl.

Particularly R^2 is thienyl.

Preferably R^3 is hydrogen.

According to one aspect of the present invention there is provided a compound of the formula **(I)** wherein:

R^1 is a group of formula **(II)** wherein R^5 is morpholino, cyclohexyl, cyclopentyl, cyclohexylmethyl and piperidino;

r is 0 or 1;

(AA^1) and **(AA^2)** are both independently selected from Phe(S), Leu(S), Phe(CH_2S), Cy(S)-Gly, Hetar(S)-Gly, alk(S)-Gly and Het(S)-Gly wherein Phe(S) and Rings A and B may be optionally substituted as hereinbefore defined and wherein the phenyl group of Phe(S) may be fused to another phenyl group to form a naphthyl group;

$\mathbf{R^2}$ is an optionally substituted 5 membered heteroaryl ring containing a maximum of four heteroatoms said optional substituents being chosen from one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, *N,N*-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, nitro, carboxy, carbamoyl,

N-(C_{1-6}alkyl)carbamoyl, N,N-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl, N-(C_{1-6}alkyl)sulphamoyl and N,N-(C_{1-6}alkyl)$_2$sulphamoyl; and

R^3 is hydrogen;

or a pharmaceutically acceptable salt thereof.

According to another aspect of the present invention there is provided a compound of the formula **(I)** wherein:

R^1 is benzyl or a group of formula **(II)** wherein R^5 is C_{1-6}alkyl (optionally substituted with a 6 membered heteroaryl ring, phenyl, phenylsulphonyl or phenoxy optionally substituted with one or more halo), C_{1-6}alkoxy, phenyl (optionally substituted with one or more halo), naphthyl or phenylC_{1-6}alkoxy;

r is 0 or 1;

AA^1 is Leu, Pyr-Ala or Phe wherein the nitrogen of the amino acid is optionally substituted with C_{1-6}alkyl;

AA^2 is Phe, Leu, Ile, Tyr, Tyr(^{t}Bu), Val, Cha, Leu(S), Phe(S) and Phe(CH_2S) and the nitrogen of the amino acid is unsubstituted and the phenyl group of Phe(S) is optionally substituted with halo, C_{1-6}alkyl or is fused to another phenyl group to form a naphthyl group;

R^2 is hydrogen, C_{1-6}alkyl [optionally substituted with C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphonyl or R^4], C_{1-6}alkoxy [optionally substituted with C_{2-6}alkynyl,] or R^4- wherein R^4 is an optionally substituted phenyl or an optionally substituted 5 membered heteroaryl ring containing a maximum of four heteroatoms said optional substituents being chosen from one or more of C_{1-6}alkyl or halo; and

R^3 is hydrogen;

or a pharmaceutically acceptable salt thereof.

A further preferred class of compounds is that of formula **(I)** wherein:

R^1 is a group of formula **(II)** wherein R^5 is methyl, ^{t}butoxy, benzyloxy or pyridylmethyl;

r is 0 or 1;

AA^1 is Leu wherein the nitrogen of the amino acid is unsubstituted;

AA^2 is Tyr, Leu or Phe wherein the nitrogen of the amino acid is unsubstituted;

R^2 is furyl, pyrazolyl (optionally substituted with one or more methyl or bromo), imidazolyl, 1,2,4-triazolyl, benzyl, methylthioethyl, isopropylthio, methoxy, isopropoxy or propynyloxy; and

R^3 is hydrogen;

or a pharmaceutically acceptable salt thereof.

Preferred compounds are those of Examples 1 - 8 or a pharmaceutically acceptable salt thereof. Especially preferred compounds are those of Examples 1-7.

Suitable pharmaceutically acceptable salts include acid addition salts such as the methanesulphonate, fumarate, hydrochloride, hydrobromide, citrate and maleate salts and salts formed with phosphoric and sulphuric acid. In another aspect suitable salts are base salts such as an alkali metal salt for example a sodium salt, an alkaline earth metal salt for example a calcium or a magnesium salt, an organic amine salt for example a salt with triethylamine, morpholine, *N*-methylpiperidine, *N*-ethylpiperidine, procaine, dibenzylamine, *N,N*-dibenzylethylamine or an amino acid for example a lysine salt. There may be more than one cation or anion depending on the number of charged functions and the valency of the cations or anions. A preferred pharmaceutically acceptable salt is a sodium salt.

Some compounds of formula **(I)** may possess chiral centres. It is to be understood that the invention encompasses all such optical isomers and diasteroisomers of compounds of formula **(I)** which possess cysteine protease inhibitory activity.

The invention further relates to all tautomeric forms of the compounds of formula **(I)**.

It is also to be understood that certain compounds of the formula **(I)** can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms.

Another aspect of the present invention provides a process for preparing a compound of formula **(I)** or a pharmaceutically acceptable salt thereof. According to this aspect of the invention there is provided a process (in which variable groups are as defined for formula **(I)** unless otherwise stated) which comprises:

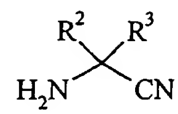
a) coupling an acid of formula **(III)**:

$$R^1\!-\!(AA^1)_r\!-\!(AA^2)\!-\!OH$$

(III)

or a reactive derivative thereof;

with an amine of formula **(IV)**:

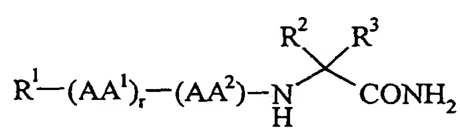


(IV)

A suitable reactive derivative of an acid of the formula **(III)** is, for example, an acyl halide, for example an acyl chloride formed by the reaction of the acid and an inorganic acid chloride, for example thionyl chloride; a mixed anhydride, for example an anhydride formed by the reaction of the acid and a chloroformate such as isobutyl chloroformate; an active ester, for example an ester formed by the reaction of the acid and a phenol such as pentafluorophenol, an ester such as pentafluorophenyl trifluoroacetate, an alcohol such as 1-hydroxybenzotriazole or a uronium salt such as 2-(1-benzotriazolyl)-1,1,3,3-tetramethyluronium hexafluorophosphate(V); an acyl azide, for example an azide formed by the reaction of the acid and an azide such as diphenylphosphoryl azide; an acyl cyanide, for example a cyanide formed by the reaction of an acid and a cyanide such as diethylphosphoryl cyanide; or the product of the reaction of the acid and a carbodiimide such as *N,N*-dicyclohexylcarbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide.

The reaction is preferably carried out in the presence of a suitable base such as, for example, an alkali or alkaline earth metal carbonate, alkoxide or hydroxide, for example sodium carbonate or potassium carbonate, or, for example, an organic amine base such as, for example, pyridine, 2,6-lutidine, collidine, 4-dimethylaminopyridine, triethylamine, morpholine or diazabicyclo-[5.4.0]undec-7-ene. The reaction is also preferably carried out in a suitable inert solvent or diluent, for example methylene chloride, acetonitrile, tetrahydrofuran, 1,2-dimethoxyethane, *N,N*-dimethylformamide, *N,N*-dimethylacetamide, *N*-methylpyrrolidin-2-one or dimethylsulphoxide, and at a temperature in the range, for example, -78° to 150°C, conveniently at or near ambient temperature.

b) dehydrating a compound of formula **(V)**:



(V)

under standard conditions.

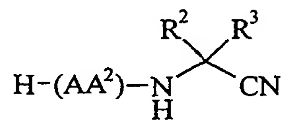
For example such a dehydration reaction may conventionally be carried out by reaction with a reagent such as trifluoroacetic anhydride. The reaction can conveniently be conducted in the presence of a suitable base as defined hereinbefore such as, for example, triethylamine. The reaction is also preferably carried out in a suitable inert solvent or diluent, as defined hereinbefore such as dichloromethane and at a temperature in the range, for example, -10°C to reflux conveniently 10°C to reflux.

c) for compounds of formula **(I)** where r = 1, coupling an acid of formula **(VI)**:

$$R^1\text{—}(AA^1)\text{—}OH$$

(VI)

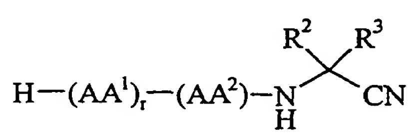
or a reactive derivative thereof as defined hereinbefore;
with an amine of formula **(VII)**:



(VII)

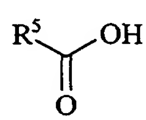
The reaction can conveniently be conducted under standard coupling conditions, such as those described in a) above.

d) For compounds of formula **(I)** where R^1 is a group of formula **(II)** reaction of an amine of formula **(VIII)**:



(VIII)

with an acid of formula **(IX)**:

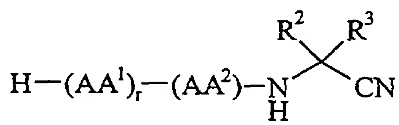


(IX)

or a reactive derivative thereof as defined hereinbefore.

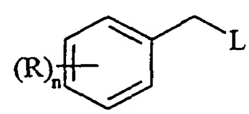
The reaction can conveniently be conducted under standard coupling conditions, such as those described in a) above.

e) Compounds of formula **(I)** where R^1 is optionally substituted benzyl may be obtained by reaction of an amine of formula **(X)**:



(X)

i) with a compound of formula **(XI)**:



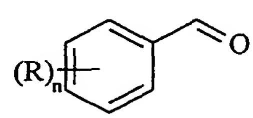
(XI)

where $(R)_n$ are optional substituents as defined above and L is a displaceable group.

A suitable displaceable group L is, for example, a halogeno or sulphonyloxy group, for example a chloro, bromo, methanesulphonyloxy or toluene-4-sulphonyloxy group.

This reaction may be carried out under standard conditions such as, for example, those described in *Synthesis* **1993**, *12*, 1243-6; or

ii) by reaction with an aldehyde of formula **(XII)**:



(XII)

This reaction may be carried out under standard conditions such as, for example, those described in *Synth. Commun.*, **1995**, *25*, *18*, 2819-2827.

If not commercially available, the necessary starting materials for the procedures described above may be made by procedures which are selected from standard organic chemical techniques, techniques which are analogous to the synthesis of known, structurally

similar compounds, by techniques which are analogous to the above described procedures or by techniques which are analogous to the procedures described in the examples.

For example, it will be appreciated that certain of the optional substituents on a phenyl or naphthyl or a heteroaryl ring in the compounds of the present invention may be introduced by standard aromatic substitution reactions or generated by conventional functional group modifications either prior to or immediately following the processes mentioned above, and as such are included in the process aspect of the invention. Such reactions and modifications include, for example, introduction of a substituent by means of an aromatic substitution reaction, reduction of substituents, alkylation of substituents and oxidation of substituents. The reagents and reaction conditions for such procedures are well known in the chemical art. Particular examples of aromatic substitution reactions include the introduction of a nitro group using concentrated nitric acid, the introduction of an acyl group using, for example, an acyl halide and a Lewis acid (such as aluminium trichloride) under Friedel Crafts conditions; the introduction of an alkyl group using an alkyl halide and Lewis acid (such as aluminium trichloride) under Friedel Crafts conditions; and the introduction of a halogeno group. Particular examples of modifications include the reduction of a nitro group to an amino group by, for example, catalytic hydrogenation with a nickel catalyst or treatment with iron in the presence of hydrochloric acid with heating; oxidation of alkylthio to alkylsulphinyl or alkylsulphonyl.

It will also be appreciated that in some of the reactions mentioned herein it may be necessary/desirable to protect any sensitive groups in the compounds. The instances where protection is necessary or desirable and suitable methods for protection are known to those skilled in the art. Thus, if reactants include groups such as amino, carboxy or hydroxy it may be desirable to protect the group in some of the reactions mentioned herein.

A suitable protecting group for an amino or alkylamino group is, for example, an acyl group, for example an alkanoyl group such as acetyl, an alkoxycarbonyl group, for example a methoxycarbonyl, ethoxycarbonyl or *t*-butoxycarbonyl group, an arylmethoxycarbonyl group, for example benzyloxycarbonyl, or an aroyl group, for example benzoyl. The deprotection conditions for the above protecting groups necessarily vary with the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl or alkoxycarbonyl group or an aroyl group may be removed for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide. Alternatively an acyl group such

as a *t*-butoxycarbonyl group may be removed, for example, by treatment with a suitable acid as hydrochloric, sulphuric or phosphoric acid or trifluoroacetic acid and an arylmethoxycarbonyl group such as a benzyloxycarbonyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-carbon, or by treatment with a Lewis acid for example boron tris(trifluoroacetate). A suitable alternative protecting group for a primary amino group is, for example, a phthaloyl group which may be removed by treatment with an alkylamine, for example dimethylaminopropylamine, or with hydrazine.

A suitable protecting group for a hydroxy group is, for example, an acyl group, for example an alkanoyl group such as acetyl, an aroyl group, for example benzoyl, or an arylmethyl group, for example benzyl. The deprotection conditions for the above protecting groups will necessarily vary with the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl or an aroyl group may be removed, for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide. Alternatively an arylmethyl group such as a benzyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-carbon.

A suitable protecting group for a carboxy group is, for example, an esterifying group, for example a methyl or an ethyl group which may be removed, for example, by hydrolysis with a base such as sodium hydroxide, or for example a *t*-butyl group which may be removed, for example, by treatment with an acid, for example an organic acid such as trifluoroacetic acid, or for example a benzyl group which may be removed, for example, by hydrogenation over a catalyst such as palladium-on-carbon.

The protecting groups may be removed at any convenient stage in the synthesis using conventional techniques well known in the chemical art.

Many of the intermediates defined herein are novel, for example, those of the formula **(V)** and these are provided as a further feature of the invention. Moreover some of the starting materials for use in process variant (b) described hereinbefore, namely those compounds of the formula **(VIII)** are not only novel but also active as inhibitors of Cathepsin L and or Cathepsin S. Accordingly these compounds are provided as a further feature of the invention.

According to a further feature of the invention there is provided a compound of the formula **(I)**, or a pharmaceutically acceptable salt thereof, for use in a method of treatment of the human or animal body by therapy.

According to a further feature of the present invention there is provided a method for producing inhibition of a cysteine protease in a warm blooded animal, such as man, in need of such treatment, which comprises administering to said animal an effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof.

The invention also provides a compound of the formula (I), or a pharmaceutically acceptable salt thereof, for use as a medicament; and the use of a compound of the formula (I) of the present invention, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in the inhibition of a cysteine protease in a warm blooded animal, such as man.

In particular the invention provides the use of a compound of the formula (I) of the present invention, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in the inhibition of Cathepsin S in a warm blooded animal, such as man.

In order to use a compound of the formula (I) or a pharmaceutically acceptable salt thereof for the therapeutic treatment of mammals including humans, in particular in the inhibition of a cysteine protease, it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

Therefore in another aspect the present invention provides a pharmaceutical composition which comprises a compound of the formula (I) or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable diluent or carrier.

The pharmaceutical compositions of this invention may be administered in standard manner for the disease condition that it is desired to treat, for example by oral, rectal or parenteral administration. For these purposes the compounds of this invention may be formulated by means known in the art into the form of, for example, tablets, capsules, aqueous or oily solutions or suspensions, (lipid) emulsions, dispersible powders, suppositories, ointments, creams, drops and sterile injectable aqueous or oily solutions or suspensions.

A suitable pharmaceutical composition of this invention is one suitable for oral administration in unit dosage form, for example a tablet or capsule which contains between 100 mg and 1 g of the compound of this invention.

In another aspect a pharmaceutical composition of the invention is one suitable for intravenous, subcutaneous or intramuscular injection.

Each patient may receive, for example, an intravenous, subcutaneous or intramuscular dose of 1 $mgkg^{-1}$ to 100 $mgkg^{-1}$ of the compound, preferably in the range of 5 $mgkg^{-1}$ to 20

mgkg^{-1} of this invention, the composition being administered 1 to 4 times per day. The intravenous, subcutaneous and intramuscular dose may be given by means of a bolus injection. Alternatively the intravenous dose may be given by continuous infusion over a period of time. Alternatively each patient will receive a daily oral dose which is approximately equivalent to the daily parenteral dose, the composition being administered 1 to 4 times per day.

The following illustrate representative pharmaceutical dosage forms containing the compound of formula **(I)**, or a pharmaceutically-acceptable salt thereof (hereafter compound X), for therapeutic or prophylactic use in humans:

Tablet I	mg/tablet
Compound X.	100
Lactose Ph.Eur.	179
Croscarmellose sodium	12.0
Polyvinylpyrrolidone	6
Magnesium stearate	3.0

(b)

Tablet II	mg/tablet
Compound X	50
Lactose Ph.Eur.	229
Croscarmellose sodium	12.0
Polyvinylpyrrolidone	6
Magnesium stearate	3.0

(c)

Tablet III	mg/tablet
Compound X	1.0
Lactose Ph.Eur.	92
Croscarmellose sodium	4.0
Polyvinylpyrrolidone	2.0
Magnesium stearate	1.0

(d)

Capsule	mg/capsule
Compound X	10
Lactose Ph.Eur.	389
Croscarmellose sodium	100
Magnesium stearate	1.

(e)

Injection I	(50 mg/ml)
Compound X	5.0% w/v
Isotonic aqueous solution	to 100%

Buffers, pharmaceutically-acceptable cosolvents such as polyethylene glycol, polypropylene glycol, glycerol or ethanol or complexing agents such as hydroxy-propyl β cyclodextrin may be used to aid formulation.

Note

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablets (a)-(c) may be enteric coated by conventional means, for example to provide a coating of cellulose acetate phthalate.

Inhibition of Cathepsin L and S.

The pharmaceutically-acceptable compounds of the present invention are useful in the inhibition of cathepsin L and cathepsin S, having a good activity *in vitro* against human cathepsin L, human cathepsin S and rabbit Cathepsin L.

Cathepsin L Assay

Recombinant human Cathepsin L was cloned and expressed in E Coli and purified using the method as described by Zeneca Limited, GB 2 306 961 A (published 14.05.1997).

Rabbit cathepsin L was purified from rabbit liver as described by Maciewicz R. A. and Etherington D. J. (Biochem. J. (1988) 256, 433-440) except the liver homogenate supernatant was concentrated by fractionation with $(NH_4)_2SO_4$ (20-80% saturation), and the pellet taken

up and dialysed against 20mM NaAcetate pH 5.5, 1mM ethylenediaminetetraacetic acid (EDTA). The supernatant was then applied to a CM Sepharose ion exchange column and cathepsin L eluted by gradient elution (0.25-0.75M NaCl). Fraction activity was determined using the synthetic substrate NCBz-Phe-Arg-NHMec as described. Cathepsin L fractions were pooled and desalted on a Sephacryl S100 column. Active fractions were pooled, adjusted to 20% saturation $(NH_4)_2SO_4$ and concentrated on a phenyl sepharose column. The remaining purification steps were as described.

Cathepsin L activity was measured based on the method of Barrett and Kirschke (1981 Methods in Enzymology, **80**, 535-561), using the fluorogenic substrates NCBz-Phe-Arg-NHMec. Inhibitors were identified by their ability to decrease the generation of the fluorescent leaving group (NHMec). Briefly the assay was as follows:

rHuman cathepsin L or rabbit cathepsin L (0.025 pmoles) was pre-incubated with or without test compound in 0.1M sodium acetate buffer pH4.5, 10mM cysteine, 0.1% Brij 35 at 25°C for 15 minutes in a solid black 96 well plate. Synthetic substrate, 20µM NCBz-Phe-Arg-NHMec, was added and the mixture incubated at 37°C for 30 minutes. The reaction was stopped by the addition of 0.1M sodium chloroacetate pH 4.3. Fluorescence was determined using a Fluoroskan II plate reader; excitation 355nm, emission 460nm. Compound potency was determined from the raw fluorescence data by calculating the IC_{50} against each enzyme using a PC graph drawing software package.

Cathepsin S assay.

Cloning and Expression of human cathepsin S.

Recombinant human cathepsin S was cloned and expressed in Baculovirus, by the following method. The cDNA sequence for human cathepsin S is available in the EMBL database Accession Number M90696. This database sequence was used to prepare, by PCR on mRNA from human tissues, a recombinant plasmid carrying an insert with a DNA sequence identical to that of cathepsin S in the EMBL database (Acc No M90696). The techniques for mRNA isolation, PCR and cloning are standard techniques known by those skilled in the art. Sequence determination of the recombinant insert was carried out using established DNA sequencing techniques.

The PCR was done so as to introduce an EcoRI cloning site 5' of the 'ATG' of cathepsin S and an XbaI cloning site 3' of the 'Stop' codon. The PCR product was cloned

between the EcoRI and XbaI sites of the baculovirus transfer vector pFASTBAC-1 (Bac-to-Bac Expression System commercially available from Gibco BRL –Life Technologies (cat no 10359-016)). This recombinant construct was used to generate, by standard techniques, a recombinant baculovirus capable of expressing preprocathepsin S.

Expression of recombinant cathepsin S was tested for the baculoviral constructs by infection of two insect cell lines : Sf9 cells (ATCC No CRL-1711) and T.ni cells (Invitrogen, Cat No B855-02).

Purification of cathepsin S

Method 1.

Procathepsin S was found in the insect cell medium and acid activated. The medium was mixed with an equal volume of 100mM Sodium Acetate buffer pH 4.5, 5mM dithiothreitol (DTT) and 5mM EDTA and incubated for one hour at 37°C method of Maubach et al (Eur. J. Biochem., 250, 745-750, 1997).

Method 2.

The pH of insect cell medium (10ml) containing procathepsin S was adjusted to 4.5 with glacial acetic acid and DTT and EDTA added to 5mM. The sample was then incubated at 37°C for 150min to enable conversion to the active enzyme. Ammonium sulphate was then added to 80% saturation and a pellet obtained by centrifugation. This pellet was redissolved in 2ml buffer A (100mM Tris, 500mM NaCl, 1mM EDTA, pH7.5) and mixed in a batchwise fashion with 100μl thiopropyl-Sepharose for 15min at 4°C. The non bound fraction was removed by a brief centrifugation and the gel washed with 2x1ml buffer A. Cathepsin S was then eluted by batch mixing with 0.4ml 20mM DTT in buffer A for 15min at 4°C.

Measurement of cathepsin S Activity.

Cathepsin S activity was measured based on the method of Maubach et al (Eur. J. Biochem., 250, 745-750, 1997), using the fluorogenic substrate Z-Val-Val-Arg-NHMec. Inhibitors were identified by their ability to decrease the generation of the fluorescent leaving group (NHMec). Briefly the assay was as follows:

rHuman cathepsin S (1.5 nmoles) was pre-incubated with or without compounds in 50mM Potassium phosphate buffer pH 6.0-6.2, 20mM Na_2EDTA, 0.1% Brij at 25°C for 5

minutes in a solid black 96 well plate. Synthetic substrate, 20μM Z-Val-Val-Arg-NHMec, was added and the mixture incubated at 30°C for 20 minutes. The reaction was stopped by the addition of 0.1M sodium chloroacetate pH 4.3. Fluorescence was determined using a Fluoroskan II plate reader; excitation 355nm, emission 460nm. Compound potency was determined from the raw fluorescence data by calculating the IC_{50} against cathepsin S using a PC graph drawing software package.

The following results were obtained on a standard *in-vitro* test system for the inhibition of Cathepsin L. The activity is described in terms of IC_{50}.

When tested in the above *in-vitro* tests the compounds of this invention give IC_{50}s in the range 1-10,000 nM.

The invention will now be illustrated by the following non-limiting examples in which, unless stated otherwise:

(i) temperatures are given in degrees Celsius (°C); operations were carried out at room or ambient temperature, that is, at a temperature in the range of 18-25°C;

(ii) organic solutions were dried over anhydrous magnesium sulphate; evaporation of solvent was carried out using a rotary evaporator under reduced pressure (600-4000 Pascals; 4.5-30 mm Hg) with a bath temperature of up to 60°C;

(iii) chromatography means flash chromatography on silica gel; thin layer chromatography (TLC) was carried out on silica gel plates; where a "Bond Elut" column is referred to, this means a column containing 10g or 20g of silica of 40 micron particle size, the silica being contained in a 60ml disposable syringe and supported by a porous disc, obtained from Varian, Harbor City, California, USA under the name "Mega Bond Elut SI";

(iv) in general, the course of reactions was followed by TLC and reaction times are given for illustration only;

(v) final products had satisfactory proton nuclear magnetic resonance (NMR) spectra;

(vi) yields are given for illustration only and are not necessarily those which can be obtained by diligent process development; preparations were repeated if more material was required;

(vii) when given, NMR data is in the form of delta values for major diagnostic protons, given in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard, determined at 250 MHz using perdeuterio dimethyl sulphoxide (DMSO-δ_6) as the solvent unless otherwise stated;

(viii) chemical symbols have their usual meanings; SI units and symbols are used;

(ix) solvent ratios are given in percentage by volume;

(x) mass spectra (MS) were run with an electron energy of 70 electron volts in the chemical ionisation (CI) mode using a direct exposure probe; where indicated ionisation was effected by electron impact (EI) or fast atom bombardment (FAB); where values for m/z are given, generally only ions which indicate the parent mass are reported;

(xi) melting points are uncorrected and (dec) indicates decomposition; the melting points given are those obtained for the materials prepared as described; polymorphism may result in isolation of materials with different melting points in some preparations; and

(xii) Z refers to benzyloxycarbonyl and Boc refers to *tert*-butoxycarbonyl.

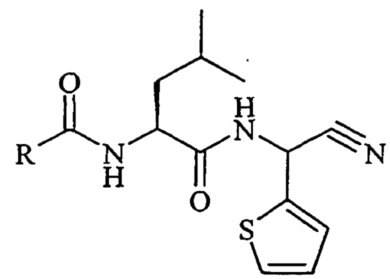
Example 1

N-(N-Morpholinocarbonyl)-(L)-leucyl-2-(2-thienyl)glycinenitrile

A mixture of N-(N-morpholinocarbonyl)-(L)-leucine (Method B) (0.323 g), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.28 g) and 1-hydroxybenzotriazole (0.197 g) in N,N-dimethylformamide (50 ml) was stirred at ambient temperature for 30 minutes. 2-(2-Thienyl)-2-aminoacetonitrile (Method A1) (0.253 g) and N-methylmorpholine (160 µl) were added and the mixture was stirred for 20 hours. The reaction mixture was evaporated to dryness (high vac) and the residue was suspended in saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate (2x50 ml). The combined ethyl acetate extracts were washed successively with 10% citric acid and brine and dried. The residue obtained on removal of the solvent was chromatographed on a Bond-elut column eluting with a mixture of ethyl acetate and dichloromethane (1/1 v/v) to give the title compound (0.159 g) as a 1/1 mixture of diastereoisomers. NMR: 9.2-9.3 (1H), 7.6 (m, 1H), 7.2 (m, 1H), 7.0 (m, 1H), 6.5 (m, 1H), 6.3 (m, 1H), 4.2 (m, 1H), 3.45 (m, 4H), 3.3 (m, 4H), 1.6 (m, 2H), 1.4 (m, 1H), 0.8 (m, 6H).

Examples 2-5

Following the method outlined in Example 1 and using the appropriate acid chlorides there were prepared:



Ex No.	R	(M+H)
2	c-Hexyl	362
3	c-Pentyl	348
4	c-Hexylmethyl	376
5	N-Piperidinyl	363

Example 6

N-[(2-Phenylacetylamino-2-phenylthio)acetyl]-2-thienylaminoacetonitrile

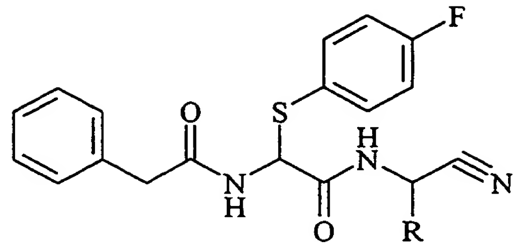
Carbonyl diimidazole (0.538g) was added to a solution of N-[(2-phenylacetylamino-2-phenylthio)acetic acid (1g) in THF (25 ml) and the mixture was stirred at 20 °C for 20 hours. 2-(2-Thienyl)-aminoacetonitrile (Method A1) (0.579 g) and triethylamine (0.664 g) were added and the mixture was stirred at 20 °C for 20 hours. The solvent was removed and the residue dissolved in dichloromethane (25 ml) and washed successively with aqueous sodium bicarbonate solution (2x20 ml) and 2M hydrochloric acid (2x20 ml). The solvent was removed and the residue was chromatographed on silica eluting with a mixture of ethyl acetate and isohexane (35:100) to give the title compound.

Diastereoisomer 1; Faster running fraction: Mp 172 °C; m/z 422 $(M+H)^+$; NMR: 9.9 (d, 1H), 8.95 (d, 1H), 7.65 (m, 1H), 7.2 (m, 11H), 6.45 (d, 1H), 5.8 (d, 1H), 3.5 (m, 2H).

Diastereoisomer 2; Slower running fraction: Mp 159 °C; m/z 422 $(M+H)^+$; NMR: 9.8 (d, 1H), 8.95 (d, 1H), 7.65 (m, 1H), 7.2 (m, 11H), 6.45 (d, 1H), 5.8 (d, 1H), 3.5 (m, 2H).

Examples 7-8

Following the method outlined in Example 6 and using the appropriate starting materials there were prepared:



Ex No.	R	M.p. °C
7 [1]	2-thienyl	144
8	H	204

[1] Mixture of diastereoisomers.

Preparation of Starting Materials

The starting materials for the Examples above are either commercially available or are readily prepared by standard methods from known materials. For example the following reactions are illustrations but not limitations of the preparation of some of the starting materials used in the above reactions.

Method A

2-(2-Furyl)-2-aminoacetonitrile

Ammonium chloride (25 g) was added to a solution of 2-furfuraldehyde (25 g) in diethyl ether (250 ml). A solution of sodium cyanide (17 g) in water (80 ml) was added over 20 minutes. The reaction mixture was stirred at ambient temperature for 14 hours , the aqueous layer was removed and the organic layer was washed twice with saturated aqueous sodium hydrogen carbonate solution (100 ml each time), dried and evaporated to dryness. The residue was dissolved in diethyl ether (250 ml) and cooled to 0 °C. Hydrogen chloride gas was bubbled through the solution keeping the temperature below 10 °C. 2-(2-Furyl)-2-aminoacetonitrile hydrochloride was filtered and dried, yield 33 g. ^{1}H NMR 6.19 (s, 1H), 6.56 (m, 1H), 6.78 (d, 1H), 7.83 (m, 1H), 9.83 (broad s, 2H).

Method A1

Following the method outlined in Method A and using the appropriate aldehyde there was prepared:

A1 2-(2-thienyl)-2-aminoacetonitrile hydrochloride

Method B

N-(N-Morpholinocarbonyl)-(L)-leucine

Lithium hydroxide solution (5.9 g in 40 ml water) was added to a solution of N-(N-morpholinocarbonyl)-(L)-leucine methyl ester (Method C) (7.3 g) in THF (40 ml) and the mixture was stirred for 20 hours. The residue obtained on removal of the solvent was suspended in water (150 ml) and washed with ethyl acetate (50 ml). The aqueous layer was acidified to pH 1 with 2M HCl and extracted with ethyl acetate (3x70 ml). The combined ethyl acetate extracts were washed with water (75 ml) and brine (75 ml) and passed through phase-separating paper. Removal of the solvent gave N-(N-morpholinocarbonyl)-(L)-leucine as an oil. NMR: 6.5 (d, 1H), 4.0 (m, 1H), 3.5 (m, 4H), 3.3 (m, 4H), 1.5 (m, 2H), 1.4 (m, 1H), 0.8 (m, 6H).

Method C

N-(N-morpholinocarbonyl)-(L)-leucine methyl ester

Triethylamine (8.4 ml) was added dropwise to L-leucine methyl ester hydrochloride (5 g) in dichloromethane (50 ml) at 0 °C followed by a solution of 4-morpholine carbonyl chloride (5 g) in dichloromethane (10 ml) and the mixture was stirred at ambient temperature for 20 hours. The reaction mixture was diluted with dichloromethane (100 ml) and washed with water (100 ml). The organic layer was collected and washed with 2M HCl (50 ml), brine (50 ml) and passed through phase-separating paper. Removal of the solvent gave N-(N-morpholinocarbonyl)-(L)-leucine methyl ester as a solid, 7.3 g. NMR: 6.7 (d, 1H), 4.1 (m, 1H), 3.6 (s, 2H), 3.5 (m, 4H), 3.3 (m, 4H), 1.6 (m, 2H), 1.4 (m, 1H), 0.8 (m, 6H); m/z: 259 $(M+H)^+$.

Method D

N-[(2-phenylacetylamino-2-phenylthio)acetic acid

This compound was prepared from phenylacetamide, glyoxylic acid and thiophenol following the procedure described in Tetrahedron, 31, 863 1975.

Method D1

Following the method outlined in Method D and using the appropriate starting material there was prepared:

N-[(2-phenylacetylamino-2-(4-fluorophenylthio)acetic acid.

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